1: X04164. Reports Bacillus subtilis...[gi:40116] Links

Comment Features Sequence

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DEFINITION  Bacillus subtilis rrnO operon promoter and leader region.
ACCESSION   X04164
VERSION     X04164.1  GI:40116
KEYWORDS    16S ribosomal RNA; inverted repeat; ribosomal RNA; rrnO operon.
SOURCE      Bacillus subtilis
  ORGANISM  Bacillus subtilis
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 353)
  AUTHORS   Ogasawara,N., Moriya,S. and Yoshikawa,H.
  TITLE     Structure and organization of rRNA operons in the region of the
            replication origin of the Bacillus subtilis chromosome
  JOURNAL   Nucleic Acids Res. 11 (18), 6301-6318 (1983)
   PUBMED   6312418
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      241 cacagcgatg tgcgtagtca gtcaaactag ggcctgcacg acgcaggtca cacaggtgtc
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VERSION     X04164.1  GI:40116
KEYWORDS    16S ribosomal RNA; inverted repeat; ribosomal RNA; rrnO operon.
SOURCE      Bacillus subtilis
  ORGANISM  Bacillus subtilis
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 6)
  AUTHORS   Ogasawara,N., Moriya,S. and Yoshikawa,H.
  TITLE     Structure and organization of rRNA operons in the region of the
            replication origin of the Bacillus subtilis chromosome
  JOURNAL   Nucleic Acids Res. 11 (18), 6301-6318 (1983)
   PUBMED   6312418
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☐ 1: X04164. Reports Bacillus subtilis...[gi:40116] Links

Comment Features Sequence

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VERSION     X04164.1  GI:40116
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SOURCE      Bacillus subtilis
  ORGANISM  Bacillus subtilis
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 6)
  AUTHORS   Ogasawara,N., Moriya,S. and Yoshikawa,H.
  TITLE     Structure and organization of rRNA operons in the region of the
            replication origin of the Bacillus subtilis chromosome
  JOURNAL   Nucleic Acids Res. 11 (18), 6301-6318 (1983)
   PUBMED   6312418
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X04164. Reports Bacillus subtilis...[gi:40116] Links

Comment Features Sequence

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VERSION     X04164.1  GI:40116
KEYWORDS    16S ribosomal RNA; inverted repeat; ribosomal RNA; rrnO operon.
SOURCE      Bacillus subtilis
  ORGANISM  Bacillus subtilis
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 6)
  AUTHORS   Ogasawara,N., Moriya,S. and Yoshikawa,H.
  TITLE     Structure and organization of rRNA operons in the region of the
            replication origin of the Bacillus subtilis chromosome
  JOURNAL   Nucleic Acids Res. 11 (18), 6301-6318 (1983)
   PUBMED   6312418
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☐ **1:** X04164. Reports Bacillus subtilis...[gi:40116] Links

Comment Features Sequence

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VERSION     X04164.1  GI:40116
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SOURCE      Bacillus subtilis
  ORGANISM  Bacillus subtilis
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REFERENCE   1  (bases 1 to 6)
  AUTHORS   Ogasawara,N., Moriya,S. and Yoshikawa,H.
  TITLE     Structure and organization of rRNA operons in the region of the
            replication origin of the Bacillus subtilis chromosome
  JOURNAL   Nucleic Acids Res. 11 (18), 6301-6318 (1983)
   PUBMED   6312418
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☐ **1:** V01490. Reports B. subtilis repli...[gi:40114] Links

Comment Features Sequence

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DEFINITION  B. subtilis replication origin region and gene rrnO start.
ACCESSION   V01490 REGION: 102..129
VERSION     V01490.1  GI:40114
KEYWORDS    16S ribosomal RNA; origin of replication; ribosomal RNA.
SOURCE      Bacillus subtilis
  ORGANISM  Bacillus subtilis
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 28)
  AUTHORS   Ogasawara,N., Seiki,M. and Yoshikawa,H.
  TITLE     Replication origin region of Bacillus subtilis chromosome contains
            two rRNA operons
  JOURNAL   J. Bacteriol. 154 (1), 50-57 (1983)
   PUBMED   6187731
COMMENT     Data kindly reviewed (12-SEP-1983) by H. Yoshikawa.
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ORIGIN
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☐ 1: V01490. Reports B. subtilis repli...[gi:40114] Links

Comment Features Sequence

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DEFINITION  B. subtilis replication origin region and gene rrnO start.
ACCESSION   V01490 REGION: 199..227
VERSION     V01490.1  GI:40114
KEYWORDS    16S ribosomal RNA; origin of replication; ribosomal RNA.
SOURCE      Bacillus subtilis
  ORGANISM  Bacillus subtilis
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 29)
  AUTHORS   Ogasawara,N., Seiki,M. and Yoshikawa,H.
  TITLE     Replication origin region of Bacillus subtilis chromosome contains
            two rRNA operons
  JOURNAL   J. Bacteriol. 154 (1), 50-57 (1983)
   PUBMED   6187731
COMMENT     Data kindly reviewed (12-SEP-1983) by H. Yoshikawa.
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ORIGIN
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Items 1 - 6 of 6

One page.

☐ **1:** D26185 Reports Links

Bacillus subtilis gene, 180 kilobase region of replication origin
gi|467326|dbj|D26185.1|BAC180K[467326]

☐ **2:** AY618310 Reports Links

Integration vector pDG3661, complete sequence
gi|49036565|gb|AY618310.1|[49036565]

☐ **3:** X04164 Reports Links

Bacillus subtilis rrnO operon promoter and leader region
gi|40116|emb|X04164.1|[40116]

☐ **4:** X04166 Reports Links

Bacillus subtilis 5S rRNA gene (3' term. of rrnO operon)
gi|40115|emb|X04166.1|[40115]

☐ **5:** V01490 Reports Links

B. subtilis replication origin region and gene rrnO start
gi|40114|emb|V01490.1|[40114]

☐ **6:** V01489 Reports Links

B. subtilis 5' end of rDNA from the 3.1Kb EcoRI fragment including 2.6Kb PstI, 1.9Kb HindIII fragments. This rrn gene set has not been mapped and has no letter designation (as do rrnA, rrnB, and rrnO of B. subtilis)
gi|40092|emb|V01489.1|[40092]

Items 1 - 6 of 6

One page.

Display Summary

Regulated Operon: *rrnO-16S-trnO-Ile-trnO-Ala-rrnO-23S-rrnO-5S*

search notes

Genes

Genes	Synonyms	Direction	Genome position	Function	COG ID
rrnO-16S		+	9809..11361	ribosomal RNA-16S	
trnO-Ile		+	11462..11538	transfer RNA-Ile	
trnO-Ala		+	11550..11625	transfer RNA-Ala	
rrnO-23S		+	11707..14634	ribosomal RNA-23S	
rrnO-5S		+	14690..14801	ribosomal RNA-5S	

Operon evidence: agrees with ribosomal RNA 5' sequence

Reference: Ogasawara N, et al. (1983), Sogin ML & Pace NR (1976)

Comments:

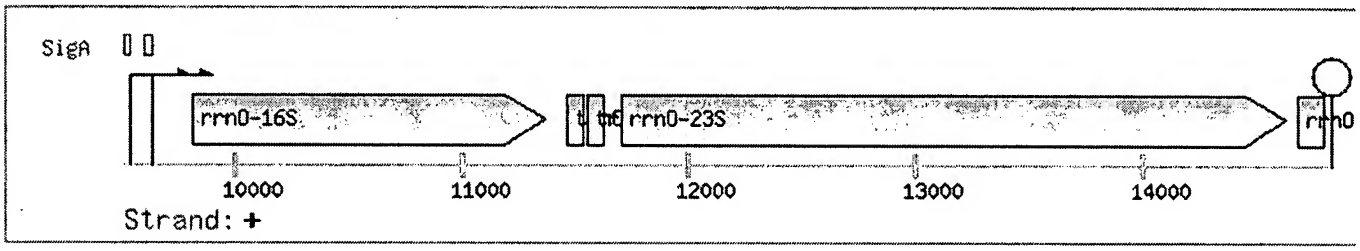
Promoters

Binding factor	Regulation	Location	Absolute position	Binding seq.(cis-element)
SigA	Promoter	-46:+17	9493..9555	TGTCATAACCCTTTACAGTCATAAAAATTATGGTATAATCATTTCTGTTGTCTTTTTA
SigA	Promoter	-45:+18	9591..9653	CAAAAAAGTATTGACCTAGTTAACTAAAAATGTTACTATTAAGTAGTCGCTTTGAGAG

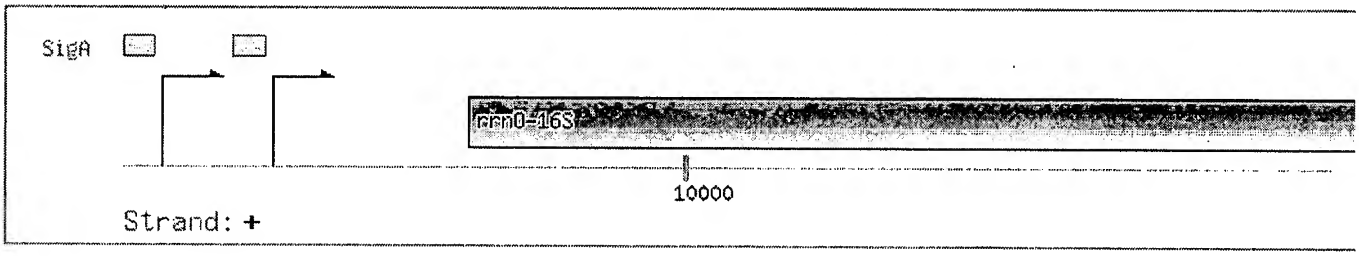
Terminator

Terminator sequence	Absolute position	Position from stop codon	Free energy [kcal/mol]	Downstream of
TTAAACCCAGCTCAATGAGCTGGGTTTTTTGTTTGTTAA >>>>>>>> <<<<<<<<	14815..14833	14..32	-15.7	rrnO-5S

Overview



Upper Region



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☐	L21	l20 and ($paraspor$ or endospor$ or spore$ or $spore).clm.
☐	L20	L19 and (strong near5 promoter)
☐	L19	L17 and promoter
☐	L18	L17 and l3
☐	L17	L16 and l13
☐	L16	L12 same (domain$ or moiet$ or fragment$ or region$ or cell-binding$ or receptor-bindin
☐	L15	L14 and (domain or moiety or fragment or region or cell-binding or receptor-binding)
☐	L14	L13 and l12
☐	L13	(bacil$ or subtil$) same ($paraspor$ or endospor$ or spore$ or $spore)
☐	L12	clostrid$ or tetan$
☐	L11	L10 and $spore
☐	L10	L9 and $toxin
☐	L9	fragment.clm. near c.clm.
☐	L8	l6 and l4
☐	L7	L6 and l5
☐	L6	l3 same promoter
☐	L5	l4 and (bacil$ or subtil$)
☐	L4	l3 and ($paraspor$ or endospor$ or spore$)
☐	L3	tttaca
☐	L2	AGAAGAACAAGAAGAAGTGTGAAAAAAAGCGCAGCTGAAATAGCTGCGCTTT
☐	L1	AGAAGAACAAGAAGAAGTGTGAAAAAAAGCGCAGCTGAAATAGCTGCGCTTT AAAATTATGGTATAATCATTTCTgTTGTCTTTTTAAAGAC

END OF SEARCH HISTORY

WEST Search History

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DATE: Wednesday, August 08, 2007

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☐	L11	L10 and (fusion or fused or link$ or coupl$ or covalent$ or join$).ti,ab,clm.	282
☐	L10	L9 and (receptor or binding or c-fragment or fragment-c or (fragment near2 c))	412
☐	L9	L8 and fragment$.ti,ab,clm.	518
☐	L8	tetan$.ti,ab,clm.	2244
☐	L7	L4 and fragment$	1
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END OF SEARCH HISTORY

DOCUMENT-IDENTIFIER: US 20050142151 A1
TITLE: Immunogenic compositions including rough phenotype Brucella host strains and complementation DNA fragments

Summary of Invention Paragraph:
[0019] Preferably, the heterologous antigen is selected from the group consisting of: Anthrax antigens such as Bacillus anthracis protective antigen (PA), inactive variants of Edema Factor and Lethal Factor; plague antigens such as Yersinia pestis F1 and V antigens and F1-V fusion proteins; malaria proteins such as circumsporozoite and merozoite antigens, and including antigens of Plasmodium berghei (sporozoite and merozoite antigens), Plasmodium falsiparum, Plasmodium vivax and Plasmodium malariae, including CSP and MSP1 antigens of all of these; Francisella antigens, particularly from Francisella tularensis; staphylococcal and streptococcal enterotoxin fragment antigens; Burkholderia antigens; Coxiella antigens; Clostridium epsilon toxoids; botulinum toxoids; smallpox antigens; mycobacterial antigens; cancer antigens; HIV antigens; tetanus toxoids (including TetC); diphtheria toxoids; pertussis toxoid; Helicobacter antigens; Borrelia antigens; Legionella antigens; Bartonella antigens; vaccinia antigens; antigen-GFP fusions; tagged antigens (6his, V5, etc.), fusions of antigens to secretory signals, fusions of antigens to each other; genes encoding therapeutic molecules or enzymes producing therapeutic molecules; antigens from other parasites, antigens from viruses, and the like. Also contemplated are heterologous genes encoding enzymes that would not serve directly as antigens but would instead synthesize non-protein products in the Brucella platform that would themselves function as heterologous antigens--for instance, lipids and polysaccharides. In addition, homologous antigens can be included to enhance immunogenicity.

Detail Description Paragraph:
[0055] Brucella, even attenuated strains, penetrate to the liver. Defense against malaria may involve destruction of liver stage parasites to prevent infection or attack on merozoites to reduce disease. Presence of antibody and Th-type 1 cellular responses, characterized by CD4 and CD8 T-lymphocytes directed at circumsporozoite protein (CSP), are associated with prevention of patent infection after challenge with P. berghei, an agent of murine malaria, and P. falciparum, which causes a severe form of human malaria. Immune response to merozoite surface protein-1 (MSP-1) of these parasites is associated with reduction in patent infection intensity. Since P. falciparum does not cause malaria in mice, the murine P. berghei model is used to demonstrate initial proof of concept for malaria vaccine approaches. Vaccines that work in the P. berghei model can be reconstructed with P. falciparum antigens. Safety and immunogenicity testing in mice and efficacy testing against blood stage infection in nonhuman primates can then lead to human trials. Department of Immunology efforts are directed toward enhancing the potency of immune response against CSP, MSP-1 and other malarial antigens. Genes for these antigens were cloned into expression vectors for use as DNA vaccines and for production of recombinant proteins for clinical vaccine tests and in vitro studies. Movement of these genes into pBBR1MCS should be readily accomplished.

Detail Description Paragraph:
[0068] As a prototype, we constructed a live, attenuated B. melitensis vaccine strain that expresses protective antigens from three known threat agents and tested it for safety, immunogenicity and protective efficacy in appropriate animal models. For instance, genes encoding some or all of the following can be used: a) protective antigen (PA) from Bacillus anthracis, b) protective C fragment of tetanus toxin and c) protective fragments of V antigen from Yersinia pestis. These antigens may be encoded by a plasmid that also encodes a Brucella gene (wboA), which, as noted above, is preferred for assisting survival of the vaccine strain in vivo and to some extent, protective immunity.

Detail Description Paragraph:

[0070] Malarial antigens are also contemplated. A Mycobacterium bovis antigen expressed on broad host range plasmid pBBR1MCS in Brucella abortus strain RB51 produced serum antibody in mice and antigen-stimulated IFN-gamma by splenocytes [Vemulapalli, Infect Immun. 68:3290-6, 2000]. Based on our own data, expressing Plasmodium antigens from plasmids in our candidate strains will likely induce strong immune responses. Defense against malaria may involve destruction of liver stage parasites to prevent infection or attack on merozoites to reduce disease. Presence of antibody and Th-type 1 cellular responses directed at circumsporozoite protein (CSP) are associated with prevention of patient infection after challenge with P. berghei, an agent of murine malaria, and P. falciparum, which causes a severe form of human malaria. The murine P. berghei model is used to demonstrate initial proof of concept for malaria vaccine approaches. Immune response to merozoite surface protein-1 (MSP-1) of Plasmodium parasites is associated with reduction in patent infection intensity. With this invention, our efforts are directed toward enhancing the potency of immune response against CSP, MSP-1 and other malarial antigens. Recombinant genes for these antigens were cloned in expression plasmids for use as DNA vaccines and for recombinant protein production in bacteria. Expression of these recombinant genes into Brucella has been accomplished using appropriate Brucella promoters. For instance, purER promoter gives low-level constitutive expression, while Kan promoter gave high constitutive expression, and groES promoter gave intermediate expression (but was inducible late). All three promoters are useful, although Kan is preferred.

CLAIMS:

1. An immunogenic composition comprising a live Brucella host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated Brucella, wherein the host cell is transformed with a recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a promoter recognizable by Brucella, and (ii) a complementation DNA fragment which is operably linked to the promoter and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell

2. The immunogenic composition of claim 1, wherein the Brucella host cell comprises a Brucella DNA fragment containing a stable non-reverting deletion mutation, having the nucleotide sequence of SEQ ID NO: 1 modified to delete nucleotides from position 1067 to position 1671.

9. The immunogenic composition of claim 1, wherein the complementation DNA fragment comprises the wboA gene.

10. The immunogenic composition of claim 9, wherein the wboA complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

11. An immunogenic composition comprising a live attenuated Brucella host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated Brucella, wherein the host cell is transformed with a recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a DNA fragment operably linked to a first promoter recognizable by Brucella, and encoding a heterologous antigen; and (ii) a complementation DNA fragment which is operably linked to a second promoter recognizable by Brucella, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell.

12. The immunogenic composition of claim 11, wherein the Brucella host cell comprises a Brucella DNA fragment containing a stable non-reverting deletion mutation, having the nucleotide sequence of SEQ ID NO: 1 modified to delete nucleotides from position 1067 to position 1671.

19. The immunogenic composition of claim 11, wherein the heterologous antigen is selected from the group consisting of anthrax antigens, Yersinia pestis F1 and V antigens and F1-V fusion proteins, malaria circumsporozoite and merozoite antigens, Plasmodium berghei antigens, Plasmodium falsiparum antigens, Plasmodium vivax antigens, Plasmodium malariae antigens, Francisella antigens, staphylococcal and streptococcal enterotoxin fragment antigens; Burkholderia antigens, Coxiella antigens, Clostridium epsilon toxoids, botulinum toxoids, smallpox antigens, mycobacterial antigens, cancer antigens, HIV antigens, tetanus toxoids, diphtheria toxoids, pertussis toxoid, Helicobacter antigens, Borrelia antigens, Legionella antigens, Bartonella antigens, vaccinia antigens, antigen-GFP fusions, tagged antigens 6his and V5, fusions of antigens to secretory signals, and genes encoding therapeutic molecules or enzymes producing therapeutic molecules.

22. The immunogenic composition of claim 19, wherein the DNA fragment of (i) encodes an enzyme synthesizes lipids and/or polysaccharides.

23. The immunogenic composition of claim 11, wherein the complementation DNA fragment comprises the wboA gene.

24. The immunogenic composition of claim 23, wherein the wboA complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

25. A vaccine against infection by brucellosis, comprising a live Brucella host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated Brucella, wherein the host cell is transformed with a recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a promoter recognizable by Brucella, and (ii) a complementation DNA fragment which is operably linked to the promoter and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell.

26. The vaccine of claim 25, wherein the Brucella host cell comprises a Brucella DNA fragment containing a stable non-reverting deletion mutation, having the nucleotide sequence of SEQ ID NO: 1 modified to delete nucleotides from position 1067 to position 1671.

33. The vaccine of claim 25, wherein the complementation DNA fragment comprises the wboA gene.

34. The vaccine of claim 33, wherein the wboA complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

36. A vaccine against infection by brucellosis and/or a non-brucellosis disease, comprising a live attenuated Brucella host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated Brucella, wherein the host cell is transformed with a recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a DNA fragment operably linked to a first promoter recognizable by Brucella, and encoding a heterologous antigen, and (ii) a complementation DNA fragment which is operably linked to a second promoter recognizable by Brucella, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell.

37. The vaccine of claim 36, wherein the Brucella host cell comprises a Brucella DNA fragment containing a stable non-reverting deletion mutation, having the nucleotide sequence of SEQ ID NO: 1 modified to delete nucleotides from position 1067 to position 1671.

44. The vaccine of claim 36, wherein the heterologous antigen is selected from the group consisting of anthrax antigens, Yersinia pestis F1 and V antigens and F1-V fusion proteins, malaria circumsporozoite and merozoite antigens, Plasmodium berghei antigens, Plasmodium falsiparum antigens, Plasmodium vivax antigens, Plasmodium malariae antigens, Francisella antigens, staphylococcal and streptococcal enterotoxin fragment antigens; Burkholderia antigens, Coxiella antigens, Clostridium epsilon toxoids, botulinum toxoids, smallpox antigens, mycobacterial antigens, cancer antigens, HIV antigens, tetanus toxoids, diphtheria toxoids, pertussis tokoid, Helicobacter antigens, Borrelia antigens, Legionella antigens, Bartonella antigens, vaccinia antigens, antigen-GFP fusions, tagged antigens 6his and V5, fusions of antigens to secretory signals, and genes encoding therapeutic molecules or enzymes producing therapeutic molecules.

47. The vaccine of claim 36, wherein the complementation DNA fragment comprises the wboA gene.

48. The vaccine of claim 47, wherein the wboA complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

50. A recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a promoter recognizable by Brucella, and (ii) a complementation DNA fragment which is operably linked to the promoter and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in a host cell transformed therewith.

51. The recombinant DNA construct of claim 50, wherein the complementation DNA fragment comprises the wboA gene.

52. A recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a DNA fragment operably linked to a first promoter recognizable by Brucella, and encoding a heterologous antigen, and (ii) a complementation DNA fragment which is operably linked to a second promoter recognizable by Brucella, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in a host cell transformed therewith.

53. The recombinant DNA construct of claim 52, wherein the complementation DNA fragment comprises the wboA gene.

54. The recombinant DNA construct of claim 52, wherein the heterologous antigen is selected from the group consisting of anthrax antigens, Yersinia pestis F1 and V antigens and F1-V fusion proteins, malaria circumsporozoite and merozoite antigens, Plasmodium berghei antigens, Plasmodium falsiparum antigens, Plasmodium vivax antigens, Plasmodium malariae antigens, Francisella antigens, staphylococcal and streptococcal enterotoxin fragment antigens; Burkholderia antigens, Coxiella antigens, Clostridium epsilon toxoids, botulinum toxoids, smallpox antigens, mycobacterial antigens, cancer antigens, HIV antigens, tetanus toxoids, diphtheria toxoids, pertussis toxoid, Helicobacter antigens, Borrelia antigens, Legionella antigens, Bartonella antigens, vaccinia antigens, antigen-GFP fusions, tagged antigens 6his and V5, fusions of antigens to secretory signals, and genes encoding therapeutic molecules or enzymes producing therapeutic molecules.

59. A method for inducing protective immunity to brucellosis in a mammal comprising the step of administering to a mammal a vaccine comprising a live Brucella host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated Brucella, wherein the host cell is transformed with a recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a promoter recognizable by Brucella, and (ii) a complementation DNA fragment which is operably linked to the promoter and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the

host cell.

60. The method for inducing protective immunity of claim 59, wherein the complementation DNA fragment comprises the wboA gene.

61. The method for inducing protective immunity of claim 60, wherein the wboA complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

63. A method for inducing protective immunity to brucellosis or a non-brucellosis disease, or both, in a mammal comprising the step of administering to a mammal a vaccine comprising a live Brucella host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated Brucella, wherein the host cell is transformed with a recombinant DNA construct replicable in Brucella, which DNA construct comprises: (i) a DNA fragment operably linked to a first promoter recognizable by Brucella, and encoding a heterologous antigen, and (ii) a complementation DNA fragment which is operably linked to a second promoter recognizable by Brucella, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell.

64. The method for inducing protective immunity of claim 63, wherein the complementation DNA fragment comprises the wboA gene.

65. The method for inducing protective immunity of claim 4964 wherein the wboA complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

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Research Interest

- **Differential Expression of Ribosomal and Transfer RNA Genes in Bacillus subtilis and**

Our research deals with the heterogeneity in organization of the conserved and redundant rRNA (rrn) operons and tRNA (trn) genes, their expression and the molecular mechanism involved in the regulation of ribosome synthesis in Bacillus subtilis. Specifically, we deal with the evolutionary importance for the endospore-forming bacteria for possessing a high clustering of rrn operons near the origin of replication and large tRNA gene clusters (2-21) downstream of all 10 ribosomal RNA gene sets. Studies on the differential expression of seven out of the 10 rrn operons with single-copy lacZ fusions capable of integrating at the native rrn site or at heterologus loci (amyE, thrC) have shown that all are under growth-rate control and are grouped into strong (**rrnO**, rrnW), intermediate (rrnA, rrnJ, rrnE), and weak (rrnD, rrnB) the latter are associated with large cluster of 16 and 21 tRNA gene clusters respectively. We are investigating the role and interaction of the tandemly arranged **promoters** (P1, P2), the Upstream Activating Sequences (UP and UAS) and the 7 bp discriminator sequence in growth-rate regulation and in the stringent control using cloned individual promoter elements without the UAS and after mutating critical regulatory regions of selected rrn operons. We follow stable RNA synthesis, ß-galactosidase measurements and (p)ppGpp accumulations in cells with genetic backgrounds that are relA+, relA-, relA(S) and rpoB grown as a function of different growth rates, during amino acid starvation or carbon source limitation.

Our second project deals with how bacterial populations specifically various Bacilli can behave in an organized manner to generate highly geometrically morphologies or morphotypes on solid and semi-solid surfaces. Five morphotypes have been generated for B. subtilis under nutrient scarcity and hardness of the agar surface: 1) the common compact round colonial growth with rough edges, (B); 2) tree branches with tip splitting growth, (T); 3) curled or chiral growth with the same handedness branches, (C); 4) vortex branched growth led by bacteria droplets that spin around a common center, (V); that 5) at times become spiral vortex (SV). These forms are stably inherited exhibiting many physiological and genetic properties distinct from B. subtilis. Sequencing of the 16S rDNA gene and Southern hybridization suggests that the colonial patterns may be the result of another Bacilli coexisting with B. subtilis which is activated during certain hostile conditions. Work is on going to understand the genetic basis of morphotypes and the role played by chemotaxis in generating and maintaining these striking differentiated structures.